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THE ROLE OF THE LYMPHATICS IN AIDING REGRESSION OF HYPOKALEMIC LESIONS IN RAT CARDIAC MUSCLE

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ABSTRACT

This study describes the role of lymphatics in the removal of macrophages from inflammatory lesions in the heart of hypokalemic rats and rats recovering from hypokalemia. The inflammatory lesions are characterized by focal cardiomyocyte necrosis, edema, and mononuclear infiltrate. The vascular and lymphatic capillaries are maintained along with the basement membrane of the necrotic cardiomyocyte. Through prior investigation, it was revealed that refeeding potassium led to a rapid reduction in lesion area. The purpose of the current investigation was to establish the role of the lymphatics as a means of reducing the lesion area by removal of the cellular infiltrate and edema. Using a limited potassium diet, hypokalemic rats were sacrificed via perfusion fixation during the hypokalemic and the potassium re-supplementation periods. Heart tissue was examined by light and electron microscopy. During the hypokalemic period, phagocytic mononuclear cells were found engulfing necrotic cardiac muscle cells. With refeeding of potassium, these phagocytic cells appeared to be diminished in number, a reduction that coincided with a decrease in the lesion size. Lymphatic channels were dilated and full of mononuclear cells. These channels were differentiated from the vascular capillaries by standard morphological criteria. In conclusion, the lymphatics play an important role in the healing process by reducing the lesion size through the removal of phagocytic cells and the uptake of proteinaceous material.

Cardiac lymphatics have been shown to be involved in removal of fluid, enzymes and other proteinaceous materials from the interstitial space of the heart (1). Various experimental edematous conditions of the heart reveal increased lymph flow. The contents of this lymph contain increased enzymes released from the damaged myocardium (2-4). Cardiac lymph vessel blockage has been shown to increase the susceptibility of the myocardium to staphylococcal valvular endocarditis and has led to myocardial fibrosis (5-7). Only limited information exists regarding cellular constituents in the lymphatics of the heart (1). Formed elements (i.e., blood cells) must come from the vascular system and enter the interstitial space prior to removal by the initial cardiac lymphatic channel. Only small quantities of these formed elements are found in the control animals' cardiac lymphatics (8,9).

This research used hypokalemic rats to investigate the role that cardiac lymphatics play in the regression of the chronic inflammatory lesion. Hypokalemia causes focal myocardial necrosis and a subsequent chronic inflammatory response (10). The response consists of chronic mononuclear inflammatory cells, frequently seen in the act of phagocytosing cell debris from degenerating or dead myocytes (11-14). The vascular and lym-

Table 1
Rat Groups and Sacrifice Times

	Group I Hypokalemia # of Rats	Group II (Dietary Control) # of Rats	Group III (Control) # of Rats
Day sacrificed			
35	3	3	3
42	3	3	3
Potassium refeeding (after Day 24)			
Every 12 hrs. for 4 days	3 (total 24)	-	-
48	3	2	2
51	3	-	-
56	3	3	3

phatic capillaries are only occasionally affected. The basal lamina surrounding necrotic myocytes also remains intact, leaving empty compartments filled with the phagocytic mononuclear cells (10). Prior morphometric investigations by this laboratory focused on the recovery from chronic nutritional hypokalemia (42 days), and revealed that the necrotic lesions' mononuclear cell infiltrate disappeared with remarkable speed following refeeding of potassium chloride. This observation was also noted by Sarkar and Levine, 1979 (15). The reduction of these lesions is most noticeable following the hypokalemic period, day 42, when potassium is resupplemented into the diet. At day 42, the lesion area includes 8.5% of the myocardium. Two and a half days after the potassium resupplementation, the lesion area had decreased to <2%. This significant reduction in lesion area ($p<0.05$) was thought to be due, in part, to the rapid removal of edematous fluid and mononuclear infiltrate. The purpose of this investigation was to establish the role of the lymphatics as a means of healing the hypokalemic lesion by promoting the removal of these mononuclear cells and edematous fluid during the injury and recovery phases.

MATERIALS AND METHODS

Sixty-four Sprague-Dawley rats weighing 200-250gms (50 to 55 days) were housed in cages with wire mesh floors to prevent consumption of bedding or fecal material containing potassium. The room was controlled for humidity and temperature which was kept at 72°F. Rats were then divided into three groups. The experimental group (Group I, n=41) underwent nutritional hypokalemia induced by a limited potassium diet (#170830) from Teklad Feed Company. To maintain the low potassium diet, drinking water was deionized. The rats received the diet for 42 days. Following the hypokalemic period, potassium was added to their diet (KCl at 150meq/L) for up to 14 days, allowing recovery. The dietary control group (Group II, n=11) consisted of rats that had received the potassium-deficient diet and KCl in their water at a 150meq/L concentration. The control group (Group III, n=11) were fed normal rat chow and tap water *(see Table 1)*.

Three rats in the dietary experimental group (Group I) were sacrificed during the potassium depletion stage on days 35 and 42. After adding potassium chloride to the water of hypokalemic animals on day 42, 3 or 4 rats were sacrificed every 12 hours for the first 4 days. Experimental group rats were also sacrificed 6, 9, and 14 days (total experimental time of 56 days) following repletion of potassium *(see Table 1)*. Three to 4 dietary control and control rats (Group II and III) were sacrificed at days 35, 42, 48, and 56 *(see Table 1)*.

Following anesthesia with sodium pentobarbital, i.p. at a dose of 35mg/Kg body weight, each animal's abdomen was opened, and the aorta and vena cava were exposed. A 22 gauge catheter was inserted into the aorta and was secured. Ringers phosphate was perfused for 3-5 minutes allowing drainage through the opening in the inferior vena cava. This was followed by fixation with 4FlG (16) for 5 minutes. Both solutions were temperature controlled at 37°C (± 2°), and pressure was controlled at 110mmHg (± 5mmHg). Tissue was then maintained in the fixative until it was processed for light and electron microscopy. Large pieces of heart cut through the right and left ventricles were embedded in paraffin, were sectioned and were subsequently examined. For electron microscopy, 1mm cubes were selected from the left myocardium and were embedded in epoxy resin. Following routine processing, the tissue was sectioned into 3-4 micron thick sections to evaluate and select for necrotic lesions. Those blocks that contained lesions were then thin sectioned, and post-stained in lead citrate and uranyl acetate. Sections were examined in either a Zeiss or Joel 100 transmission electron microscope and were analyzed qualitatively with particular focus on the lymphatic capillaries and ducts.

The following were accepted criteria for lymphatics of perfusion fixed heart tissue. The lymphatic lumens should contain electron dense proteinaceous material, while the blood capillary lumens are free of electron dense fluid and cells due to the washout by the perfusate (17,18). Lymphatic endothelial cells are thin and delicate. They contain few pinocytotic vesicles and parallel microfilaments (18,19). Discontinuous basal lamina surrounds the lymphatic vessel, and anchoring filaments extend from the adluminal surface of the endothelial cells to connect with surrounding cells or connective tissue (19,20). Another differentiating factor is that the lymphatic endothelial cells nuclei protrude into the lumen of the lymphatic vessel (17,18). Employing these observations, a functional description of the lymphatics' activity was examined during a chronic type of inflammation and recovery.

RESULTS

During the development of the cardiac lesions in the Group I rats, myocytes underwent focal degeneration and necrosis. Within and around these necrotic foci, mononuclear infiltrates were revealed *(Fig. 1)*. Mononuclear cells were seen in the interstitium and were observed invading the basal lamina of the cardiac myocytes *(Fig. 2)*. They were also found in lymphatic channels. There was no endothelial damage, or denudation of endothelial cells in either the blood or lymphatic capillary vessels. The capillary spaces were patent in both of these types of vessels *(Fig. 1, 3, and 4)*. The lymphatic lumens contained electron dense proteinaceous material, while the blood capillary lumens were clear of proteinaceous material and cells due to the washout by the Ringer's solution and fixative *(Fig. 4)*. Lymphatic endothelial cells were narrow. They contained cytoplasmic filaments of 5-7u thickness and few calveolae *(Figs. 5 and 6)*. Discontinuous basal lamina surrounded the lymphatic space. Anchoring filaments were attached to the external surface of the endothelial cells and extended to connect with surrounding collagen or reticular fibrils *(Fig. 7)*. Nuclei of the endothelial cells protruded into the lumen of the lymphatic channels whereas the vascular capillaries did not *(Fig. 4)*. Neither of the control groups, Groups II or III, developed lesions *(see Table 2)*.

Phagocytic cells surrounded by an electron dense gray infiltrate were revealed in the lymphatics during the hypokalemic and the early recovery phases (12-72 hours after repletion of potassium) *(Figs. 3 and 4)*. Lymphatic capillaries were filled with phagocytic cells that contained features characteristic of macrophages, i.e., secondary lysosomes containing myocyte debris *(Fig. 4)*. These cells were found within the luminal space of lymphatic vessels *(Figs. 3 and 4)*. The lymphatic vessel itself showed no evidence

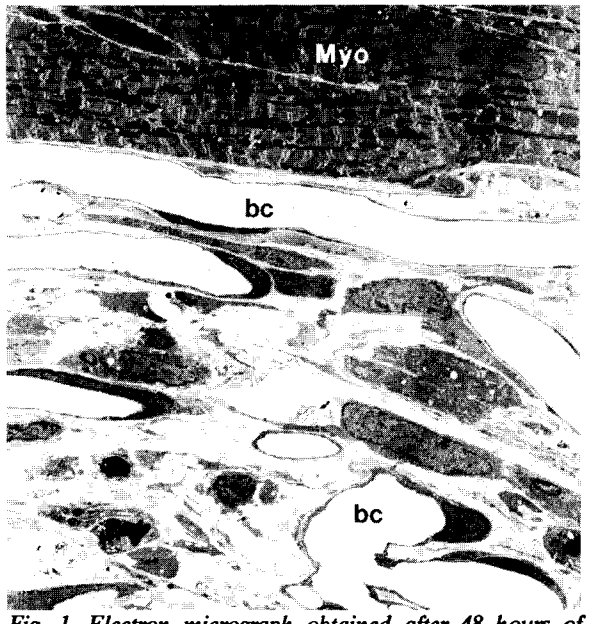

Fig. 1. Electron micrograph obtained after 48 hours of potassium repletion of characteristic myocardium at top (Myo) and an area of focal necrosis induced by the 42 day hypokalemic period. Myocytes have degenerated or are removed, mononuclear infiltrate, and patent blood capillaries (bc) are revealed (original magnification x1200).

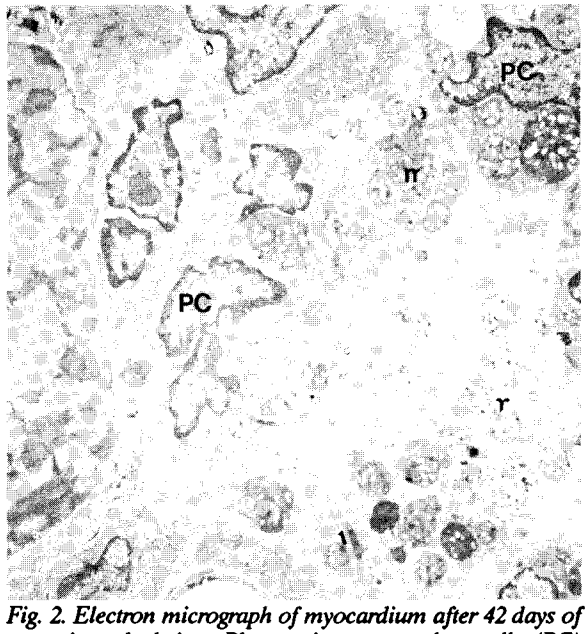

Fig. 2. Electron micrograph of myocardium after 42 days of potassium depletion. Phagocytic mononuclear cells (PC) invading beyond the basal lamina of a necrotic cardiac muscle cell and engulfing mitochondria (m) and debris (original magnification x3150).

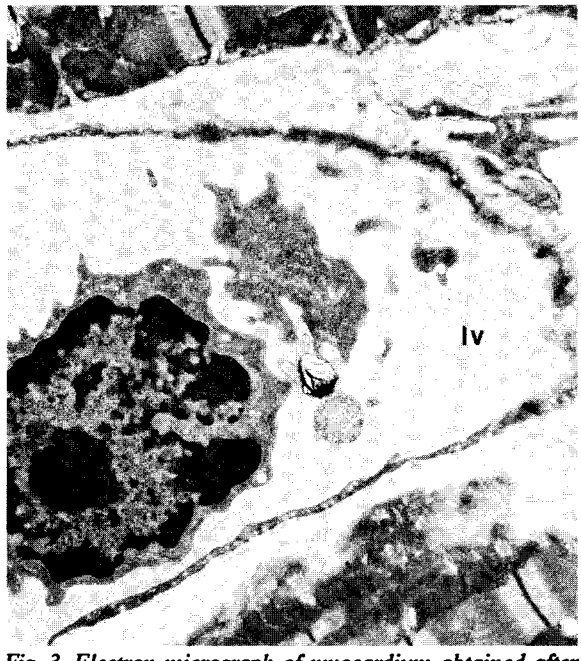

Fig. 3. Electron micrograph of myocardium obtained after 36 hours of potassium repletion. A cardiac lymphatic vessel (lv) contains a mononuclear cell and electron dense material in the lumen (original magnification x20,000).

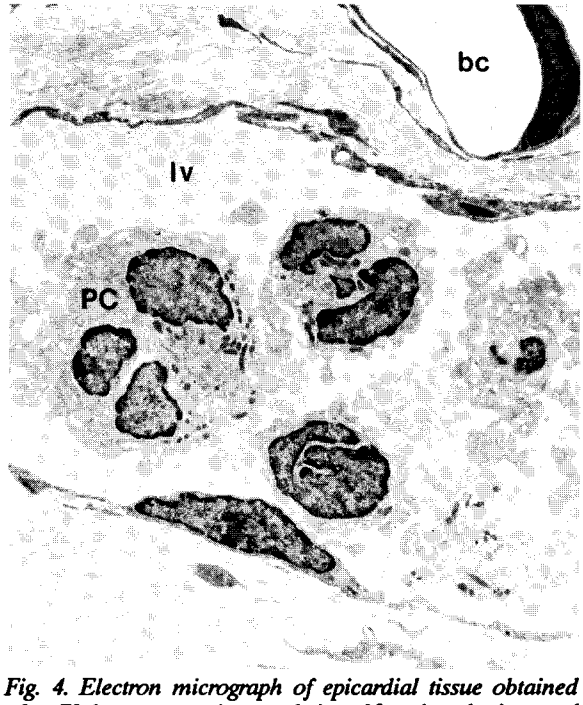

Fig. 4. Electron micrograph of epicardial tissue obtained after 72 hours potassium repletion. Note lymphatic vessel (lv) filled with phagocytic cells (PC) and homogenous grey infiltrate. The lymphatic endothelial nucleus bulges into the lumen of the vessel while the blood capillary (bc) endothelial nucleus does not obstruct the lumen (original magnification x3000).

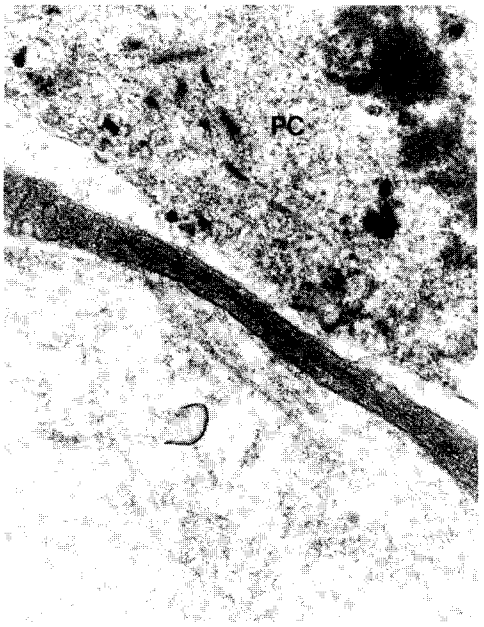

Fig. 5. Electron micrograph of myocardial lymphatics after 36 hours potassium repletion. Mononuclear phagocytic cell (PC) in the lymphatic lumen. Note intra-cytoplasmic filaments in the lymphatic endothelial cell (original magnification x31,500).

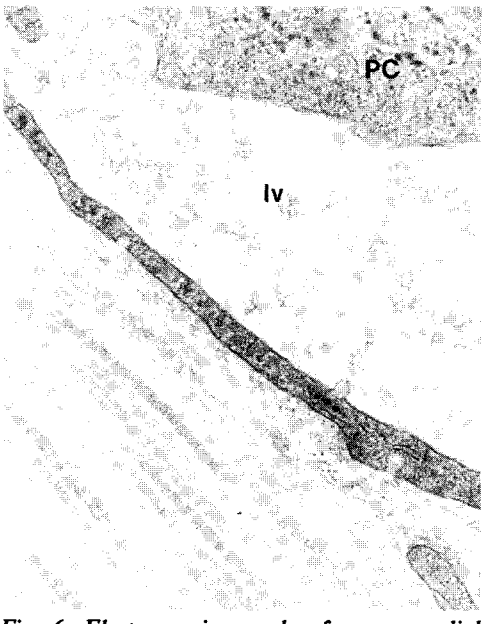

Fig. 6. Electron micrograph of a myocardial lymphatic endothelial cell after 36 hours potassium repletion. Mononuclear phagocytic cell (PC) in the lymphatic vessel (lv). Lymphatic endothelial cell with few vesicles (original magnification x31,500).

of active phagocytosis or cell degeneration. During the early recovery period, there were fewer mononuclear cells in the interstitial space. This coincided with a decreased necrosis of cardiac myocytes. The figures revealing lymphatics with cellular infiltrate *(Figs. 3-7)* were taken from the early recovery period, 12 to 72 hours after potassium resupplementation.

During later recovery (84 hours onward), lymphatic vessels filled with mononuclear infiltrate were not observed. Also, lymphatic vessels from either of the control groups did not reveal any cellular infiltrate in their lumens.

DISCUSSION

This study documented a cellular infiltrate within the heart's lymphatic system in hypokalemic and in recovering hypokalemic rats. For a cellular infiltrate to occur in these vessels, the cells must have entered by way of the interstitial space (1). Since the lymphatics themselves were not damaged, a lymphangitis was ruled out. Therefore, the cells found within the lymphatic vessels were hypothesized to be related to the mononuclear infiltrate invading the focally necrotic myocardium. Support for this include: 1) the secondary lysosomes, seen in some of the cellular infiltrate in the lymph vessels, contained what appeared to be myocyte debris (mitochondria with flocculent densities), and 2) the control group's lymphatic vessels did not contain any cells. While these mononuclear cells were found in lymphatic channels of both the hypokalemic and the recovering Group I rats, it should be understood that some focal lesions were occurring in animals maintained on a dietary hypokalemic regimen while other lesions were regressing. Consequently, in the hypokalemic rats (Group I, days 35 and 42) and the recovering hypokalemic rats (Group I, after day 42), the cells located in the lymphatic channels were

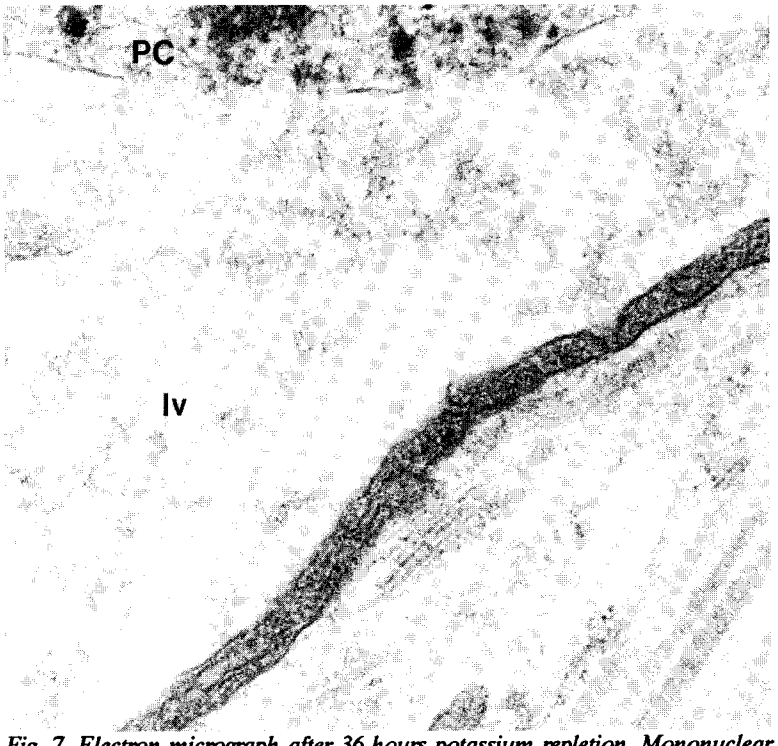

Fig. 7. Electron micrograph after 36 hours potassium repletion. Mononuclear phagocytic cell (PC) in the lymphatic vessel (lv). Lymphatic endothelial cell with anchoring filament attached to adjacent collagen fibers (original magnification x31,500).

thought to be coming from areas where the focal necrosis was regressing. This removal process possibly allowed immunological recognition by transmission of information and by stimulation of immunocompetent cells in the nearby lymph

Table 2
Summary of Results

	Lesions	Mononuclear Infiltrate	Cells in Lymphatics
Group I			
Dietary Experimental			
Hypokalemic Period	+ +	+ +	+ +
Early Recovery (12-72 hours)	+	+	+ +
Late Recovery (84 hours onward)	-	-	-
Group II			
Dietary Control	-	-	-
Group III			
Control	-	-	-

(+ +) - greatest amount, ↑; (+) - decreased amount but > control; (-) - control values

nodes (21,22).

Investigations employing cannulated cardiac lymphatics reported primarily red cells and limited white blood cell populations in control lymph (8,9). These studies also disclosed no increase in the cellular components with the experimental induced abnormalities investigated. Although not specifically stated, neither disturbance (experimental congestive heart failure or a 60 minute ligation of the coronary sinus) led to a significant cellular infiltrate within the interstitium of the heart. It was this distinction that made this study different from its predecessors. While our study revealed a mononuclear cellular infiltrate in the lymphatic channels, it was highly unlikely that this represented merely control numbers of white blood cells. In fact, multiple lymphatic vessels filled with cellular infiltrates were not found in the control rats (Groups II and III). Since the cell populations in the cardiac lymphatic channels were similar to those of infiltrates in nearby focal lesion, they probably arose from these cell populations. In our study, there were no red blood cells in the lymphatics in contrast to reports by Uhley, et al. (8) and Leeds, et al. (9). With experimental ischemia produced in dogs by Fjeld, et al. (3), a 300-500% increase of red blood cells in the lymph of the experimental group over the control values was seen within 60 seconds of experimental hypoxia. The latter authors postulated the hypoxic period induced enough damage to allow red blood cells to emigrate into the interstitial space and to be taken up by the initial lymphatic vessel. Although cellular infiltrate was increased in their animals, the cell type varied in relation to the pathological process in the heart.

With chronic pathological situations, as observed in the present investigation, the lymphatics were filled not only with mononuclear cells but also with an electron dense material. This "debris" most probably was related to the proteinaceous edema which surrounded the focal lesions. Initial lymphatic capillaries were designed for the uptake of such material, since they have loose intercellular junctions, a discontinuous basal lamina, overlapping endothelial cells, and anchoring filaments which aid in the patency of the lumen during various pathological conditions (23-25).

While other studies have revealed that the blockage of the lymphatic vessels leads to a prolongation of healing and susceptibility of disease (5,7), this investigation has concluded that the maintenance of lymphatics during the pathological incident allows for a rapid recovery by carrying away the cellular infiltrate and edematous fluid. This verified objective morphological measurements which revealed the dynamics of the drainage function of these lymphatics, i.e., the myocardium was restored to control values within six days, following a 42-day dietary hypokalemic period.

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